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TITLE: Combinational Targeting of Prostate Carcinoma Cells and Tumors Associated Pericytes with Antibody Based Immunotherapy and Metronomic Chemotherapy

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14. ABSTRACT
The hybridoma secreting the HMW-MAA-specific mAb 225.28 which is used for immuno prevention of prostate carcinoma and the hybridoma secreting the isotype matched mAb F3C25 have been tested for activity. Ascitis has been prepared and monoclonal antibodies have been purified and monitored for purity and activity. The colony of TRAMP mice has been expanded to test the efficacy of mAb 225.28 plus cyclophosphamide metronomic therapy in the inhibition of progression of prostate cancer. Sixty-four TRAMP mice have been enrolled in the combinatorial treatment schedule. Animals are being screened 2 times a week for palpable tumors.

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Table of Contents

Combinatorial targeting of prostate cancer cells and tumor associated pericytes with antibody-based immunotherapy and metronomic chemotherapy.

PROGRESS REPORT

INTRODUCTION

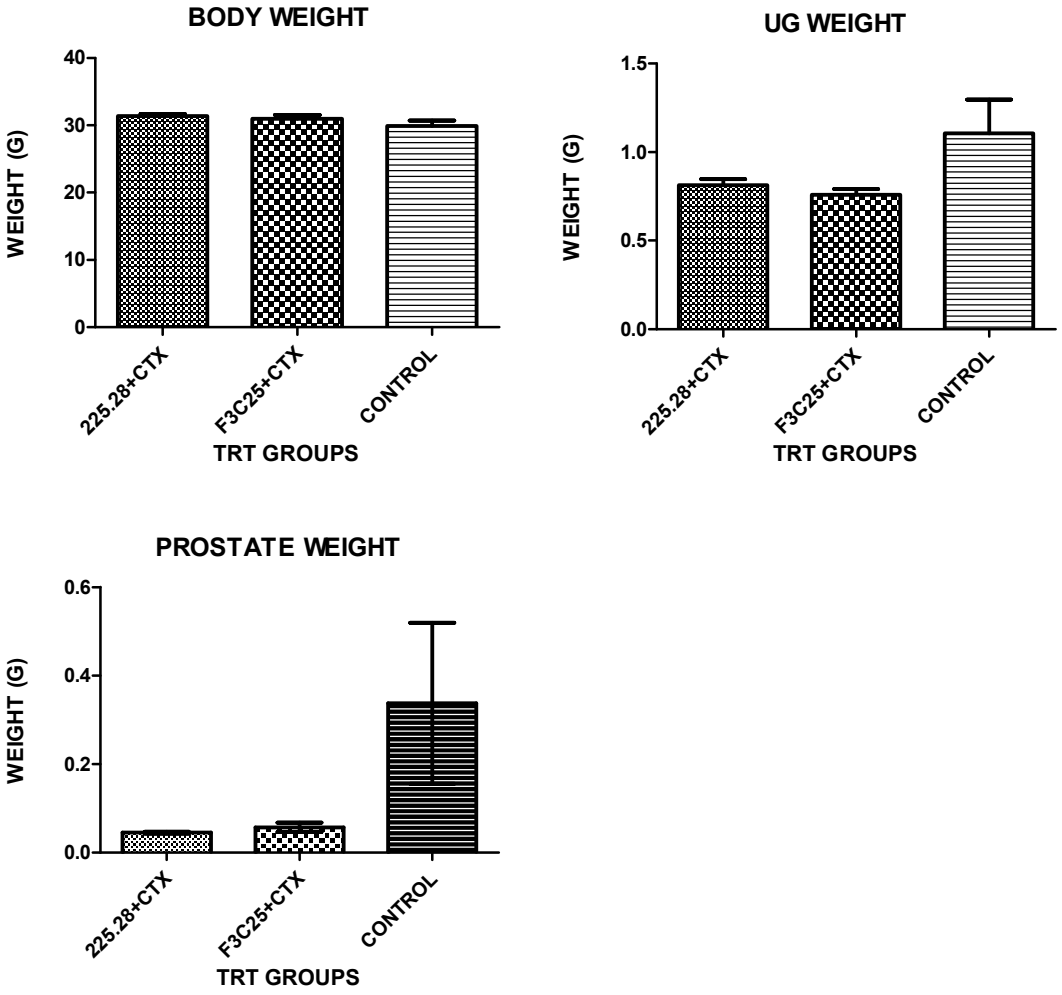
The lack of efficacy of convention al therapies in pros tate cancer has stim ulated interest in d eveloping and implementing novel therapeutic strategies. Am ong them is immunotherapy. To i mmunize the negative im pact of escape m echanisms on the outcom e of i mmunotherapy, the present proposal aim s at showing that the efficacy of immunotherapy of prostate can cer can be enhan ced by targ eting no t on ly cancer cells, bu t also activated pericytes in th e tum or m icroenvironment with a combinat orial immunotherapy. The latter includes AN-2-specific m onoclonal antibody and continuous ad ministration of low dos e cyclophospham ide, i.e. metronomic chemotherapy.

BODY

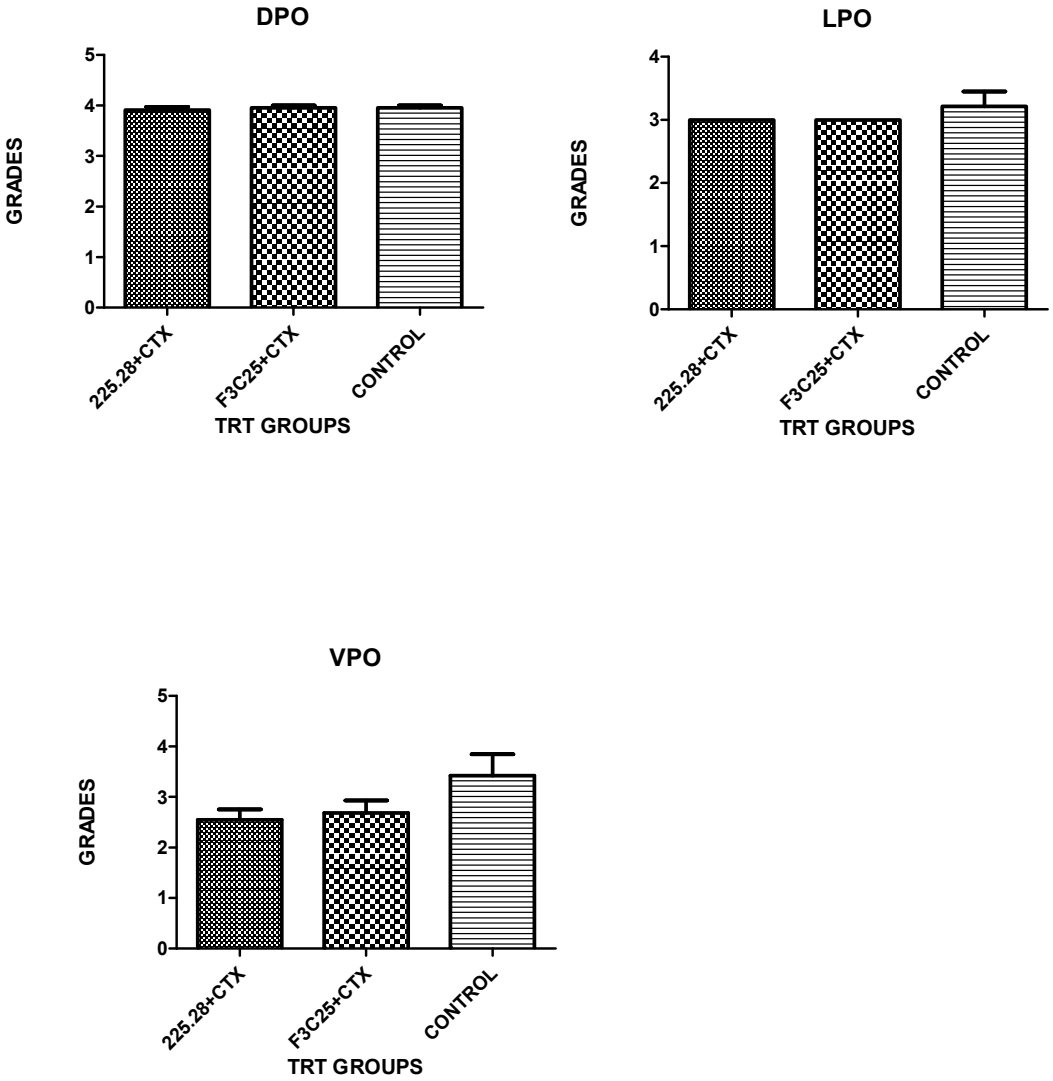
Delay of tumor progression in TRAMP m ice by com binatorial targeting of prostate cancer c ells and activ ated pericytes in the tum or m icroenvironment with AN2-specific mAb in com bination with CTX m etronomic chemotherapy. TRAMP mice were bred and enrolled on treatment starting at 10 weeks-of-age. Mice in cohort 1 **(225.28 + CTX,** n=27 **)** received i.p. injections of AN2- specific purified m Ab (225.28) at 100µg/injection/mouse in com bination with oral administration of m etronomic chemotherapy of cyclophosphomide (CTX) at a dose of 10 mg/kg/day in drinking the water th roughout the experiment. Mice in cohort 2 **(F3C25 + CTX**, n=26**)** received i.p. injections of purified irrelevant isot ype matched mAb (F3C25) at 100µg/injection/mouse in com bination with oral administration of m etronomic chemotherapy of cyclophosphomide at a dose of 10 mg/kg/ day in drinking the water throughout the experiment. Mice in cohort 3 (**Control**, n=24) were left untreated as a reference for the na tural course of th e disease. Ani mals were observed 3 times a week (MWF) for signs of toxicity a nd tumor formation. Antibody injections w ere given on Tuesdays and Fridays. At 18 weeks-of-age the anim als were euthanized and prostatic tissu es and relevant data collected. Body weights, reprodu ctive tract weights and prostatic weights were collected. (**Graph 1**) There was no difference in the body weights be tween the different cohor ts indicating that trea tment did not adversely impact the overall vigor of the anim als. The UG and pr ostate weights were decreased in th e treated anim als compared to contro l with a trend towards im proved response with c ombinational therapy as m easured by prostate weight. The prostatic com plex was m icrodissected under a stereom icroscope and the individual lobes of the prostate were weighed. The com bination of dorsa l (DP), latera l (LP) and ve ntral (VP) prosta te weig ht was combined to give the prostate we ight. Prostate tissues were for malin fixed and e mbedded in paraffin and processed for histological evaluation by hematoxylin and eosin staining. Histology was evaluated and each lobe of the pro state was given a tum or grade. Grad e 1 represents normal prostate with secretory epithelial cells and an open lumen. Grade 2 has an open lum en but areas for transformed cells with a s hift in the c ytoplasmic to nuclear ratio and a piling up of the cells. In grade 3 all the epithelial cells have a transformed phenotype and the lumen is being filled with cells and a profound piling of the cells. Grad e 4 represen ts carcinoma in situ with cells invading through the basem ent m embrane. Grade 5 represent solid tum ors wi th a glandular architecture

and grade 6 are poorly differentiated tumors with sheets of anaplastic cells. Each lobe of the prostate was given three tumor grades representing the best grade, the worst grade and the overall grade. **Graph 2** is the overall grade for the dorsal, lateral and ventral prostate. The ventral prostate was examined more in depth because it is has been shown to be the most responsive to therapeutic intervention and demonstrated the most response in this study. Graphs of the initial analysis of the data for tumor grade in the VP are presented in **Graphs 3 & 4**. The distribution of tumor grade was examined by low, intermediate and cancer. Low represents grades 1 and 2. Intermediate represents grade 3. Cancer presents grades 4, 5 and 6. The data for distribution is presented in **Table I and Graph 4**.

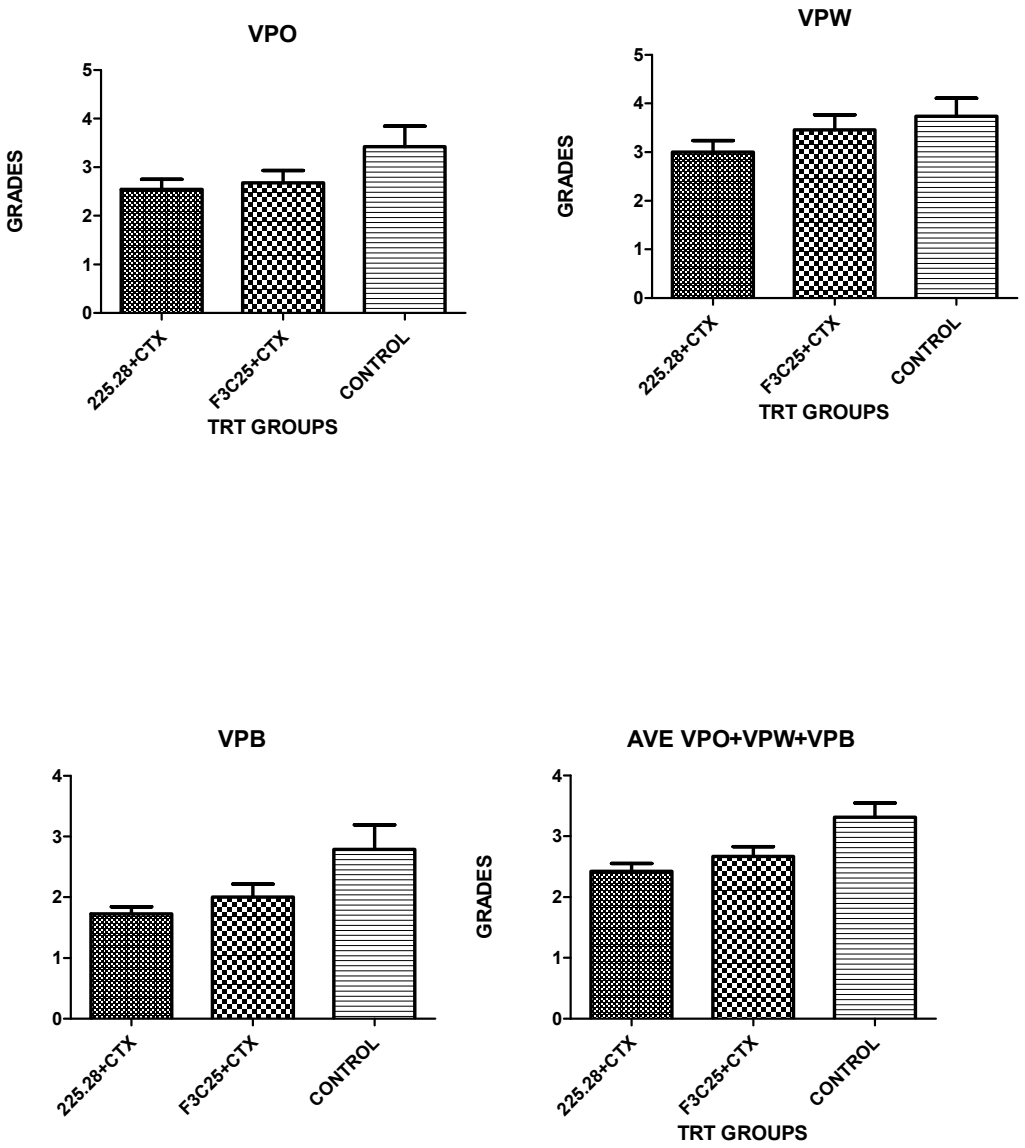
Additional slides have been cut and are being stained to examine the endothelial cells and pericytes of the prostatic vessels.



Graph 1. Body weight, UG weight and prostate weight of each cohort. Note that there is a trend towards further decrease in prostate weight in the combination therapy (225.28 + CTX). Bars are Standard Error



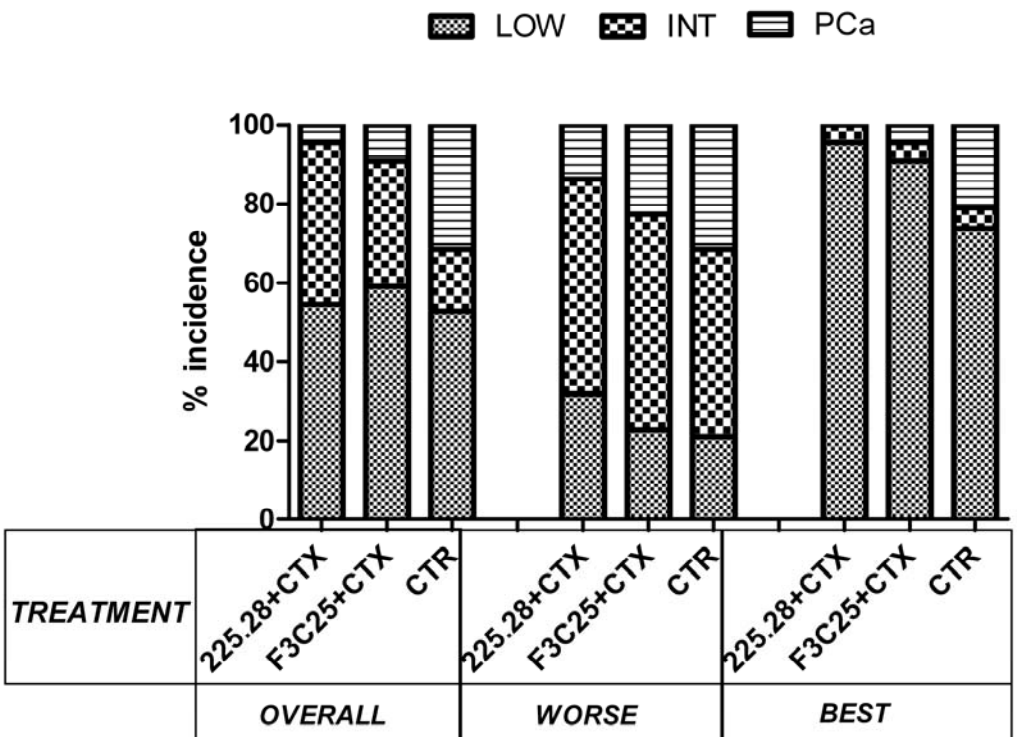
Graph 2. Overall tumor grade of dorsal prostate (DP), :Lateral Prostate (LP) and ventral prostate (VP). Note that in the ventral prostate, which has been demonstrated to be the most responsive lobe of the prostate, there is a trend towards decreased tumor grade. Bars are Standard Error



Graph 3. Ventral Prostate Grades Overall (VPO), Worse (VPW), Best (VPB) and Average (VPO+VPW+VPB). Note the trend towards improved response with the combination therapy. Bars are Standard Error

Table I. Tumor Grades for Ventral Prostate

Grade VP overall	225.28 + CTX	F3C25 + CTX	Control
1	1	0	0
2	11	13	10
3	9	7	3
4	0	0	0
5	0	0	0
6	1	2	6
Grade VP worse	225.28 + CTX	F3C25 + CTX	Control
1	0	0	0
2	7	5	4
3	12	12	9
4	1	0	0
5	0	0	0
6	2	5	6
Grade VP best	225.28 + CTX	F3C25 + CTX	Control
1	7	5	2
2	14	15	12
3	1	1	1
4	0	0	0
5	0	0	0
6	0	1	4



Graph 4. Grade distribution for Ventral Prostate. Note that with treatment the prostate have lower grade with less incidence of cancer. There is a trend for improved response with combinational treatment versus CTX with control antibody.

KEY RESEARCH ACCOMPLISHMENT

- Treatment of twenty seven TRAMP male mice with AN2-specific mAb and with metronomic chemotherapy and of twenty six TRAMP m ice with m etronomic ch emotherapy has shown that both treatments delay tumor progression. Studies are in pr ogress to determ ine whether the re sults of the two treatm ents are significantly different.

REPORTABLE OUTCOMES

Administration of AN2-specific mAb with metronomic chemotherapy and of metronomic chemotherapy delays disease progression.

CONCLUSION

The results obtained thus far indicate th at the therap eutic stra tegy w e outlined in the gra nt applic ation is effective. Experim ents are in progress to dete rmine whether the com bination AN2-specific m Ab a nd metronomic chem otherapy is m ore effective than metronomic chemotherapy alone. Furtherm ore the mechanisms underlying these effects have to be investigated.